IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Serial No:	09/593,914
Date Filed:	June 14, 2000
Application Title:	Probes, Probe Sets, Methods And Kits Pertaining To The Detection, Identification And/or Enumeration Of Yeast; Particularly In Wine
Applicants:	Hyldig-Nielsen et al.
Group Art Unit:	1634
Examiner:	Carla Myers
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Appeal Brief Cover

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

APPEAL BRIEF FOR APPELLANTS

Jens J. Hyldig-Nielsen
Heather P. O'Keefe
Henrik Stender

Probes, Probe Sets, Methods And Kits Pertaining To The Detection, Identification And/or Enumeration Of Yeast; Particularly in Wine

Serial No. 09/593,914
Filed: June 14, 2000
Group Art Unit: 1634
Appeal No.:

The attached Appeal Brief is submitted in accordance with the notice mailed on August 22, 2003. A petition under 37 C.F.R. § 1.136 for an automatic 4-month extension of time is being filed herewith thereby making this Appeal Brief due on February 22, 2004. All other appropriate fees for the Appeal Brief have been paid. A request for an Oral Hearing, and payment of the appropriate fee accompanies this Appeal Brief. No other fees are believed to be due for the filing of this paper but if The Office disagrees, The Office is authorized to deduct the appropriate fee from Deposit Account 02-3240.

Respectfully submitted,

February 18, 2004
Date:

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Appeal Brief (in triplicate)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re the application of: Appeal No.

Hyldig-Nielsen et al. Examiner: Carla Myers

Serial Number: 09/593,914 Group Art Unit: 1634

Filed: June 14, 2000

For: Probes, Probe Sets, Methods And Kits Pertaining To The Detection, Identification And/Or Enumeration Of Yeast; Particularly In Wine

BRIEF ON APPEAL

February 18, 2004

INTRODUCTION

This is an appeal from the action of the Examiner dated February 25, 2003, finally rejecting claims 1-8, 10-12, 16, 18, 19, 21-26, 29, 32, 24, 46-49, 61, 62 and 80-87, all of the claims pending in this application. The Examiner has maintained a restriction requirement with respect to claims 10, 11, 21, 22, 34, 61 and 62. Appellants did traverse the restriction requirement, did provide appropriate and timely filed arguments in response to the restriction requirement as well as a timely filed petition under 37 C.F.R. § 1.144 on August 22, 2003 requesting the withdrawal of the restriction requirement.

In addition, claims 1-8 and 46 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Kosse (reference DF) in view of Stender (1998; WO98/15648; reference BB). Claims 47-49 and 80-85 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Kosse (reference DF) in view of Stender (1998; WO98/15648; reference BB) and in further view of Parton (US 5,905,038). Claims 1-8, 10-12, 16, 18-19, 21-26, 29, 32, 33, 46, 61-62, 86 and

87 stand rejected under 35 U.S.C. §103(a) as being unpatentable over De Wachter (GeneBank Accession No. x58052) in view of Kosse (reference DF) in further view of Stender (1998; WO98/15648; reference BB). Claims 47-49 and 80-85 stand rejected under 35 U.S.C. §103(a) as being unpatentable over De Wachter in view of Kosse (reference DF) and Stender (1998; WO98/15648; reference BB) in further view of Parton (US 5,905,038). A Notice of Appeal was timely filed on August 22, 2003. A petition under 37 C.F.R. § 1.136(a) for an automatic four-month extension of time is being filed with this brief. Accordingly, it is believed that this Brief is due on or before February 22, 2004 and is therefore being timely filed. Accordingly, please consider this Brief On Appeal.

1. REAL PARTY IN INTEREST

The application has been assigned to Boston Probes, Inc., 15 DeAngelo Drive, Bedford, MA. USA. As of the filing of this Brief On Appeal, Boston Probes, Inc. is wholly owned by Applera Corporation, acting through its Applied Biosystems stock group. These stand as the parties having an interest herein.

2. STATEMENT OF RELATED APPEALS AND INTERFERENCES

No related appeals and/or interferences are pending.

3. STATUS OF CLAIMS

Claims 1-8 and 46 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Kosse (reference DF) in view of Stender (1998; WO98/15648; reference BB). Claims 47-49 and 80-85 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Kosse (reference DF) in view of Stender (1998; WO98/15648; reference BB) and in further view of Parton (US 5,905,038). Claims 1-8, 10-12, 16, 18-19, 21-26, 29, 32, 33, 46, 61-62, 86 and 87 stand rejected under 35 U.S.C. §103(a) as being unpatentable over De Wachter (GeneBank Accession No. x58052) in view of Kosse (reference DF) in further

view of Stender (1998; WO98/15648; reference BB). Claims 47-49 and 80-85 stand rejected under 35 U.S.C. §103(a) as being unpatentable over De Wachter in view of Kosse (reference DF) and Stender (1998; WO98/15648; reference BB) in further view of Parton (US 5,905,038). No claim stands allowed.

4. STATUS OF AMENDMENTS

All amendments of record appear to have been entered. The claims set forth in Section 9 (Appendix) reflect the entry of all amendments. No new amendments are offered.

5. SUMMARY OF THE INVENTION

The present invention pertains to probes, probe sets, methods and kits for the detection, identification and/or enumeration of yeast; particularly in wine (Title).

Accordingly, in some embodiments, the invention pertains to enzyme-linked probes suitable for use in an *in-situ* hybridization assay wherein the probes comprise a probing nucleobase sequence directed to a target sequence within yeast. (Specification at page 15, lines 26-28). In some embodiments, the enzyme-linked probe can be suitable for detecting, identifying or quantitating *Dekkera/Brettanomyces*, and particularly *Dekkera bruxellensis* yeast, in a sample of interest. (Specification at page 16, lines 12-14). Preferred probing nucleobase sequences of said yeast specific probes are listed in Table 1. (Specification at page 16, lines 16-17)

In some embodiments, the invention pertains to probe sets suitable for detecting, identifying or quantitating *Dekkera/Brettanomyces in-situ*, and particularly *Dekkera bruxellensis* yeast, in a sample of interest. (Specification at page 17, lines 20-22) Preferred probing nucleobase sequences of said yeast specific probes are listed in Table 1. (Specification at page 17, lines 22-24)

In some embodiments, the invention pertains to a method for detection,

identification or quantitation of yeast using enzyme-linked probes in an *in-situ* hybridization (ISH) assay. (Specification at page 19, lines 32-34). The method comprises contacting one or more species of yeast with a yeast specific enzyme-linked probe, under suitable *in-situ* hybridization conditions, to thereby form a probe/target sequence hybrid. (Specification at page 19-20, bridging sentence). The enzyme activity can then be used to detect, identify or quantitate yeast present in the sample. (Specification at page 19-20, bridging sentence). In some embodiments, the method is applied to the determination of *Dekkera/Brettanomyces* yeast. (Specification at page 20, lines 10-27)

In some embodiments, the invention pertains to kits for performing an *in-situ* assay that detects, identifies or enumerates *Dekkera/Brettanomyces*, and particularly *Dekkera bruxellensis* yeast, in a sample of interest. (Specification at page 23, lines 30 to page 31, line 28)

In some embodiments, the invention pertains to methods, kits and compositions that are particularly useful for the rapid *in-situ* probe based detection, identification and quantitation of slow growing yeast. (Specification at page 22, lines 11-13). Said assays can typically be completed within 27-48 hours. (Specification at page 23, lines 24-25).

6. ISSUES ON APPEAL

Issue 1:

The Examiner issued a restriction requirement in the Office Action dated July 18, 2001. In a response dated January 17, 2002, Appellants responded to the restriction requirement by making an election as required by the rules of practice and submitting arguments supporting a traverse of the restriction requirement. Appellants submitted a timely petition under 37 C.F.R. § 1.144 on August 22, 2003 requesting withdrawal of the restriction requirement and reiterating the arguments of record. Appellants have not been informed as to the

outcome of said petition. Assuming that the restriction requirement is not withdrawn as a result of the timely filed petition, Appellants believe the restriction requirement is improper and therefore seek withdrawal of the restriction requirement by The Board. For brevity, The Board is requested to review the arguments set forth in the petition under 37 C.F.R. 1.144 file on August 22, 2003.

Issue 2:

Claims 1-8, 10-12, 16, 18, 19, 21-26, 29, 32, 34, 46-49, 61, 62, 80-87 stand pending in the application. All pending claims have been rejected under 35 U.S.C. § 103(a) in view of various combinations of references. No claim stands allowed.

All rejections under 35 U.S.C. § 103(a) are respectfully traversed. The issue on Appeal is whether or not each of these pending rejections is proper. Appellants request that The Board overrule the Examiner and withdraw the rejections.

7. GROUPING OF CLAIMS

Because there are four distinct rejections under 35 U.S.C. § 103(a) that remain pending, it is believed that at least four claim groups (Groups I-IV) exist. It is believed that within certain of the groups, there are subgroups that exist based upon specific arguments set forth below. Accordingly it is believed that the grouping and subgrouping of the claims is as follows:

Group I, Claims 1-8 and 46 based upon the combination of Kosse (reference DF) with Stender (1998; WO98/15648; reference BB);

Group II, Claims 47-49 and 80-85 based upon the combination of Kosse (reference DF) with Stender (1998; WO98/15648; reference BB) in further view of Parton (US 5,905,038);

Group III, Claims 1-8, 10-12, 16, 18-19, 21-26, 29, 32, 46, 61-62, 86 and 87 based upon the combination of De Wachter (GeneBank Accession No.

x58052) with Kosse (reference DF) in further view of Stender (1998; WO98/15648; reference BB);

Group IV, Claims 47-49 and 80-85 based upon the combination of De Wachter (GeneBank Accession No. x58052) with Kosse (reference DF) and Stender (1998; WO98/15648; reference BB) in further view of Parton (US 5,905,038).

Subgroups **B** and **A** include those rejections based upon the De Wachter reference and those that do not, respectively. Thus, Subgroup A includes Groups I and II and Subgroup B includes Groups III and IV.

8. APPELLANT'S ARGUMENTS

I. Statement Of The Facts

Appellants reproduce below, for the convenience of the Board, such facts and evidence as support certain of the forthcoming arguments. Appellants note that facts and evidence are a product of the record. The record includes the specification as filed as well as each of the references considered during prosecution.[1] The source for each fact is noted with emphasized text indicated in bold, as appropriate.

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1 See: *In re Wiseman*, 596 F.2d 1019, 1023, 201 U.S.P.Q. 658, 661 (C.C.P.A. 1979) where the court looks to the specification for support regarding Appellant's claim to unexpected results. Moreover, the court in *In re Schulze* looked to the specification for support of a claim to unexpected results by stating: "Nor do we find anything in the record by way of **disclosure** (emphasis added) or affidavit ..." (*In re Schulze*, 52, C.C.P.A. 1422, 1424, 346 F.2d 600, 602, 145 U.S.P.Q. 716, 718 (C.C.P.A. 1965) Furthermore, "In determining whether the invention as a whole would have been obvious under 35 U.S.C. § 103, we must first delineate the invention as a whole. In delineating the invention as a whole, we look not only to the subject matter which is literally recited in the claim in question (the ratio of values) but also to those properties of the subject matter which are inherent in the subject matter and are **disclos d in the specification** (emphasis added)." *In re Antonie*, 559 F.2d 618, 619, 195 U.S.P.Q. 6, 8 (Fed. Cir. 1977). Finally, what a reference teaches is a **question of fact**. *In re Bell*, 991 F.2d 781, 784, 26 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1993).

A. FACTS ESTABLISHED BY THE SPECIFICATION

Fact 1:

"However, the use of enzyme-labeled DNA probes for the detection of yeast cells by *in-situ* hybridization has not yet been demonstrated (Amann, R. I., Zarda, B., Stahl, D.A. and Schleifer, K.-H., **Identification of individual prokaryotic cells by using enzyme-labeled, rRNA-targeted oligonucleotide probes**, *Applied and Environmental Microbiology, 58:* 3007-3011 (1992)) and Applicants are unaware of any attempts to use enzyme-labeled PNA probes to detect yeast by *in-situ* hybridization. The lack of examples of successful ISH assays utilizing enzyme linked probes likely results because of difficulties in getting such large molecules to pass through the cell membrane into the yeast cytoplasm." (Emphasis original; Specification at pages 3-4 bridging paragraph).

B. ADMISSIONS/STATEMENTS BY THE EXAMINER

Fact 2:

"The reference (Kosse) teaches that prior to *in situ* hybridization, yeast cell walls **must** be permeabilized and that probes **must** be selected to yeast 18S rRNA regions which are fully accessible to probes (see page 478)." (Emphasis and annotations added; Office Action dated February 25, 2003 at page 3)

Fact 3:

"Kosse teaches that the *in situ* hybridization method is performed using fluorescent-labeled probes and that dot blot hybridization is performed using digoxigenin labeled probes. Kosse **does not** specifically teach using enzyme-linked probes, or specifically soy bean peroxidase labeled probes." (Emphasis added; Office Action dated February 25, 2003 at page 3)

Fact 4:

The Examiner has argued: "However, as stated in the office action of Paper

No. 13, there are no teachings in Amann which indicate that the enzyme linked probes cannot be applied to the detection of yeasts. Amann provides the results obtained when applying the enzyme-linked probes to the detection of S. cerevisiae. However, Amann does not teach that these results apply to the detection of all yeasts" (Office Action dated February 25, 2003 at page 4)

C. FACTS ESTABLISHED BY AMANN ET AL., APPLIED AND ENVIRONMENTAL MICROBIOLOGY, 58(9): 3007-3011 (1992);

REFERENCE CA

Fact 5:

"Hitherto we had not achieved penetration of enzyme-labeled probe into gram positive bacteria **or yeast cells**." (Emphasis added; Amann et al., at the Abstract).

Fact 6:

"The molecular weight of horseradish peroxidase (40,000) is approximately 100 times greater than that of fluorescein or tetramethylrhodamine, **the two most common labels of rRNA-targeted oligonucleotide probes for single-cell identification. This increases the overall molecular weight of a probe molecule from approximately 6,000 to about 50,000, and penetration of enzyme-labeled probe through the cell periphery might be expected to hinder whole-cell identification**. However, we were encouraged by our previous studies demonstrating detection of DIG-labeled oligonucleotides with HRP-labeled anti-DIG Fab fragments (22). These conjugates have a molecular weight of at least 100,000, but nevertheless probe-conferred, HRP-mediated intracellular formation of a substrate precipitate could be demonstrated for gram-negative cells after lysozyme-EDTA treatment. Thus, it was not surprising that an enzyme-probe conjugate could also penetrate all strains of gram-negative bacteria examined in this study. But again this was effective only after lysozyme-

EDTA treatment. **On th other hand, the gram-positive bacteria xamined (*B. subtilis* and several strains of lactococci) remained impermeable to HRP-labeled probe even after prolonged incubation with lysozyme, lysostaphin (Sigma), or mutanolysin (Sigma) at different concentrations.** Following extensive digestion, the gram positive bacteria were only partly strained while gram negative bacteria, added as a control, were lysed (data not shown). Proteinase K (Boehringer Mannheim) treatment was effective but could not be controlled in a way to preserve morphological integrity of the cells. They could hardly be visualized by phase-constant microscopy and hybridized weakly probably because of loss of target molecules (data not shown)." (Emphasis added; Amann et al., at page 3008-3010, bridging paragraph).

Fact 7:

"We encountered similar problems with cells of *Saccharomyces cerevisiae*, and again attempts to make the intracellular RNA accessible by enzymatic pretreatment (lyticase and β–glucoronidase; both from Sigma) of the cells or detergent addition **failed**." (Emphasis added; Amann et al., at page 3010, col. 1).

Fact 8:

"**For now, enzyme-labeled oligonucleotides can therefore be used only for specific detection and identification of gram-negative bacteria and several members of *Euryarchaeota* (21).**" (Emphasis added; Amann et al. at page 3010, col. 1).

Fact 9:

"We are continuing to evaluate alternative approaches to permeabilize whole fixed cells. **Although we are optimistic** that methods can be tailored for single strains under investigation, we do not expect to find a universal method that will permeabilize the whole array of different microorganisms to a

comparable degree. This means that general probes (e.g., for the three domains) should not be used as enzyme derivatives for the characterization of environmental samples because they will likely produce biased results. Nevertheless, highly specific enzyme-probe conjugates used in combination with suitable methods to permeabilize specific cells of interest should provide a valuable tool for the detection and identification of individual cells *in situ.* (Emphasis added; Amann et al. at page 3010, col. 1 to col. 2, bridging paragraph).

D. FACTS ESTABLISHED BY THE DECLARATION OF DR. HENRIK STENDER[2]

In his Declaration of August 21, 2003 (Appendix B), Dr. Stender made the following statements now entered as Facts:

Fact 10:

"I am a co-inventor of PCT/DK97/00425 (WO98/15648) entitled: "Novel Probes For The Detection Of Mycobacteria", (herein either Stender (1998; WO98/15648; reference BB) or Stender (1998)) referred to by the Examiner in the above captioned application (09/593,914) as Stender (1998) and am familiar with its contents" (Annotation added; Stender Declaration at page 2)

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2 In the Advisory Action dated September 29, 2003, the Examiner acknowledges that she refused to consider the Declaration of Dr. Stender stating that it raises new issues and was not timely filed. With all due respect, the issue of what Amann and his colleagues teach has been raised by Appellants from as far back as the Specification (**Fact 1**). What Amann and the other references teach is a matter of fact and has been repeatedly treated in the prosecution history of this application. Accordingly, the statement that the Declaration of Dr. Stender raises new issues appears to be evasive and inaccurate. Accordingly, Appellants believe the Declaration of Dr. Stender was properly filed and should have been considered because the Examiner has continuously misinterpreted the actual teachings of the references as well as their impact on the impressions of the ordinary practitioner with respect to the requirements of an obviousness determination under 35 U.S.C. § 103(a).

Fact 11:

"I have reviewed Kosse et al., Systems. Appl. Microbiol. 20: 468-480 (1997)" (Stender Declaration at page 2)

Fact 12:

I have reviewed Amann et al., Applied and Environmental Microbiology 58(9): 3007-3011 (1992)" (Stender Declaration at page 2)

Fact 13:

"Based upon my review of the Amann et al. reference, I believe that: 1) Amann et al. teach that it was well accepted, at the time of their publication, that enzyme-linked (labeled) probes COULD NOT readily penetrate the cell wall of yeast; 2) Amann et al. had no success with getting enzyme-labeled probes into yeast; and 3) Amann et al. would tend to dissuade one of skill in the art from attempting to use an enzyme-linked probe to analysis [sic] a yeast in an in-situ based assay" (Annotation added; Stender Declaration at page 2-3, bridging paragraph)

Fact 14:

"Amann et al. has, to my knowledge, never published a successful permeabilization method for yeasts for in situ analysis with enzyme-labeled probes" (Stender Declaration at page 3)

Fact 15:

"Kosse et al. would not, in view of Amann et al., tend to motivate the application of enzyme-linked probes to in-situ assays for yeasts because the reference does not address this particular assay format" (Stender Declaration at page 3)

Fact 16:

"Stender (1998) would not, in view of Amann et al. tend to motive the application of enzyme-linked probes to in-situ assays for yeasts because the reference does not address yeasts" (Stender Declaration at page 3)

Fact 17:

"One of skill in the art at the time of the present invention would not have a reasonable expectation of successfully applying enzyme-linked probes to the determination of yeast in an in-situ based assay because there was inadequate teaching available as to how to permeablize the cell wall of the yeast to these large molecules." (Stender Declaration at page 3)

E. FACTS ESTABLISHED BY KOSSE ET AL., SYSTEM. APPL. MICROBIOL. 20: 468-480 (1997)

Fact 18:

"Therefore, the applicability of whole cell hybridization with **fluorescently labeled oligonucleotide probes** for the identification of eucaryotic yeasts from dairy products was investigated." (Emphasis added; Kosse et al. at page 469, col. 1)

Fact 19:

"The first step for hybridization of whole yeast cells to oligonucleotide probes is the **permeabilization** of the cell wall which might prevent penetration of the probes." (Emphasis added; Kosse et al. at page 474 col. 1)

Fact 20:

"Prior to in situ hybridization, yeast cell walls **must be permeabilized**." (Emphasis added; Kosse et al. at page 478, col. 1)

Fact 21:

"Experiments with a variety of species-specific fluorescently labeled oligonucleotides revealed that **only the 3'-end of 18S rRNA is accessible for species specific probes**." (Emphasis added; Kosse et al. at page 474, col. 1) It is noted that Figure 3 of Kosse et al. indicates that only positions 1682-1728 are "accessible for species-specific probes".

Fact 22:

"Therefore, we take our results as evidence that the target regions of these probes on the yeast 18S rRNA **were not fully accessible**." (Emphasis added; Kosse et al. at page 478, col. 2)

Fact 23:

"The **restricted accessibility** of potential target sites on 18S rRNA for species-specific probes currently limits the applicability of fluorescence-*in situ*-hybridization for yeasts. In these cases, alternative hybridization techniques based upon the extraction of nucleic acids must be used, e.g., the dot blot technique with DIG-labeled probes." (Emphasis added; Kosse et al. at page 478, col. 2)

F. FACTS ESTABLISHED BY STENDER (1998; WO98/15648; REFERENCE BB)

Appellants have presented the following as Facts at page 8-9 of the Office Action response dated December 6, 2002. Because the Examiner has apparently not taken issue with any of the foregoing, it is believed that no dispute exists as to these facts.

Fact 24:

"Stender (1998) does not teach anything about yeasts but is limited to determinations of mycobacteria."

Fact 25:

"Stender (1998) teaches enzyme-linked probes for the determination of mycobacteria but does not teach about permeabilizing yeast cells to enzyme-linked probes."

II. The Law Of 35 U.S.C. § 103(a)

1. General Standard:

"A claimed invention is unpatentable if the differences between it and the prior art "are such that the subject matter **as a whole** (emphasis added) would have been obvious at the time the invention was made to a person of ordinary skill in the art." *In re Dembiczak*, 175 F.3d 994, 998, 50 U.S.P.Q.2d 1614, 1616 (Fed. Cir. 1999). In determining whether a claimed invention is obvious one must consider; 1) the scope and content of the prior art; 2) the level of skill in the prior art; 3) the differences between the claimed invention and the prior art; and 4) objective evidence of non-obviousness such as secondary factors. Id.

The PTO bears the burden under 35 USC § 103 to establish an unrebutted *prima facia* case of obviousness. *In re Rouffet*, 149 F.3d 1350, 1355, 47 U.S.P.Q.2d 1453, ___ (Fed. Cir. 1998). To satisfy its burden, the PTO must show some objective teaching in the prior art or that knowledge generally available in the art would lead the ordinary practitioner to combine **relevant teaching**. *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598 (Fed. Cir. 1988). In the absence of a proper *prima facia* case of obviousness, an Applicant is entitled to a patent. *In re Rouffet*, 149 F.3d 1350, 1355, 47 U.S.P.Q.2d 1453, ___ (Fed. Cir. 1998). To overcome a claimed *prima facia* case of obviousness, an Applicant can either show that the *prima facia* case of obviousness is

insufficient because it relies on incorrect factual predicates or otherwise present secondary evidence of non-obviousness. *Id.*

A proper rejection under 35 USC § 103 may not be premised upon "bald assertions" for which there is no support for or explanation of a conclusion. *Id.* An Examiner's cursory statement unaccompanied by evidence or reasoning is entirely inadequate to support a rejection. *In re Sichert*, 566 F.2d 1154, 1164, 196 U.S.P.Q. 209, 217 (C.C.P.A., 1977). A rejection based on section 103 must be based in fact that is not aided by hindsight. *In re Warner*, 54 C.C.P.A. 1628, 1635, 379 F.2d 1011, 1017, 154 U.S.P.Q. 173, 178 (C.C.P.A., 1967). The PTO may not resort to speculation, unfounded assumptions or hindsight reconstruction to supply deficiencies in its factual basis for a rejection. *Id.* Doubts as the factual basis for a rejection must to resolved in favor of the Applicant since it is the PTO's burden to establish a *prima facia* case of obviousness. *Id.*

2. Application of Hindsight:

On the permissibility of an Examiner's use of hindsight, the law is quite clear. Recent courts have held that: "Our analysis begins in the text of section 103 quoted above, with the phrase "at the time the invention was made". For it is this phrase that guards against the "tempting but forbidden zone of hindsight,"." *In re Dembiczak*, 175 F.3d 994, 998, 50 U.S.P.Q.2d 1614, 1617 (Fed. Cir. 1999). Measuring the claimed invention against the standard established by section 103 requires the oft-difficult but critical step of casting the mind back to the time of invention, to consider the thinking of one of ordinary skill in the art, guided only be the prior art references and **the then-accepted wisdom in the field** (emphasis added)." *Id.* Close adherence to this methodology is especially important to avoid using the inventor's own teachings against the teacher. *Id.* The best defense against application of hindsight is **rigorous application** of a requirement for a showing of the teaching or motivation to combine the prior art references. *Id.* The examiner can satisfy this burden only by showing some objective teaching

leading to the combination. *Id.* That showing must be **clear and particular**. *Id.* "Broad conclusory statements regarding the teaching of multiple references, standing alone, are not "evidence." *Id.* The mere fact that the prior art may be modified in a manner suggested by the Examiner does not make the modification obvious unless the prior art expressly suggested the modification. *In re Fritch*, 972 F.2d 1260, 1266, 23 U.S.P.Q.2d 1780, 1783-1784 (Fed. Cir. 1992). Moreover, when prior art references require selective combination, there must be some reason for the combination other than the hindsight gleaned from the invention itself. *Interconnect Planning Corp. v. Feil*, 774 F.2d. 1132, 1143, 227 U.S.P.Q. 543, 551 (Fed. Cir. 1985). The Examiner may not pick and choose among isolated disclosures to deprecate the claimed invention. *In re Fritch*, 972 F.2d 1260, 1266, 23, U.S.P.Q.2d 1784, 1784 (Fed. Cir. 1992).

3. Obvious To Try Is Not The Standard Of Obviousness

"Obvious to try" is not a legitimate test of patentablility. *In re Fine,* at 1075, 5 U.S.P.Q.2d 1596,1599 (Fed. Cir. 1988). Admonition that the subject invention is "obvious to try" is generally directed to two types of error. In one case (motif one), that which would be "obvious to try" is to vary all parameters or to try each of numerous possible choices until one arrives at a successful result, where the prior art gave no indication of which parameters where critical and no directions as to which of many possible choices were likely to be successful. In the other case (motif two), that which is "obvious to try" is to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it. *In re O'Farrell*, 853 F.2d 894, 903, 57 USLW 2147, ___, 7 U.S.P.Q.2d 1673, 1681 (Fed. Cir. 1988).

4. Expectations of Success

Both suggestion and reasonable expectation of success must be found in

the prior art *and not in Applicant's disclosure. In re Vaeck*, 947 F.2d 448, 493, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991). Moreover, a prior art disclosure should be considered as much for how the teachings differ with the subject invention as well as how they are similar. *Id.* at 494, 20 U.S.P.Q.2d 1438, 1443.

III. Arguments Supporting Patentability

a) All Of The Present Rejections Are Prima Facia Deficient

As noted above, the burden rests with The Office, and specifically the Examiner, to, at a minimum, establish a *prima facia* case for obviousness else the Applicants (herein the Appellants) are entitled to a patent. To establish a *prima facia* case for obviousness the Examiner state the **motivation** to combine relevant art. The mere fact that the prior art may be modified in a manner suggested by the Examiner does not make the modification obvious unless the prior art expressly suggested the modification. *In re Fritch*, 972 F.2d 1260, 1266, 23 U.S.P.Q.2d 1780, 1783-1784 (Fed. Cir. 1992).

It is respectfully submitted that upon careful review of each of the rejections appearing at paragraphs 3, 4, 5, or 6 of the Office Action dated February 25, 2003, it is clear that the Examiner has failed to describe any particular motivation to combine the references. In each case, the Examiner simply asserted that it was *prima facia* obvious to combine the references to thereby achieve the presently claimed subject matter. Such a rejection merely takes elements from various references and combines them using the Appellant's specification as a blueprint to achieve a description of the claimed subject matter. Such a rejection is hindsight based and clearly prohibited by precedent such as *In re Dembiczak*, 175 F.3d 994, 50 U.S.P.Q.2d 1614 (Fed. Cir. 1999).

For these reasons it is believed that each and every rejection under 35 U.S.C. § 103(a) must properly be withdrawn and all presently pending claims be allowed. In particular this applies to all claims being rejected based upon the arguments set forth in paragraphs 3, 4, 5 and 6 of the Office Action dated

February 25, 2003. In particular this applies to the claims in Subgroups A & B, Groups I, II, III and IV.

b) The combination of Kosse with Stender is Improper

Each and every one of the presently pending rejections requires the proper combination of Kosse (reference DF) with Stender (1998; WO98/15648; reference BB). In addition to lacking a proper motivation to combine the references, it is respectfully submitted that the Examiner has committed the following additional errors:

1) The art is non-analogous;
2) There is no reasonable expectation of success;
3) The art teaches away from the claimed invention;
4) Obvious to try is not the standard under 35 U.S.C. § 103(a).

It is well accepted that any rejection based upon 35 U.S.C. §103(a) must rely upon analogous art. M.P.E.P. § 2141.01(a). It is respectfully submitted that Stender (1998; WO98/15648; reference BB) is non-analogous art.

All of the presently pending claims pertain to the methods, kits and composition suitable for the in-situ detection, identification and/or enumeration of **yeast**. Whilst Kosse (reference DF) pertains to the determination of yeast with fluorescently labeled probes (**Fact 3, 15, 18**), Stender (1998; WO98/15648; reference BB) pertains to the determination of mycobacteria, not yeast (**Facts 10, 16, 24 & 25**). It is well established that to perform an *in-situ* assay on yeast, the cell walls of the yeast must be permeable to the probe (**Facts 1, 2, 6, 17, 19 & 20**) else no determination can be made. There is no evidence presented by the Examiner that the references in any way teach that mycobacteria are in any way related to yeast so that permeabilization techniques applied to mycobacteria can be, or might be, successfully applied to yeast. Many Facts suggest that no such conclusion can be drawn (e.g. **Facts 7, 8, 13, 14, 16 and 17**). Accordingly it is believed that Stender is non-analogous art and for at least this reason, it is not

properly combined with Kosse.

It is well accepted that in addition to motivation to combine references, the references themselves must provide a reasonable expectation of success in the practice of the elements as combined. That expectation must be clear from the teaching of the references and not opined by the Examiner. Appellants reiterate that Stender (1998) pertains to the determination of mycobacteria, not yeast (**Facts 10, 16, 24 & 25**). Kosse pertains to the determination of yeast with fluorescently labeled probes (**Fact 3, 15, 18**) and by the Examiner's own statements, Kosse doe not teach, disclose or even suggest the use of enzyme-linked probes (**Fact 3**) or methods for the permeabilization of cell wall of yeast to these large molecules. Because there is not a single reference that teaches the ordinary practitioner about how to permeabilize the cell walls of a yeast so as to enable an enzyme-linked probe to effectively interact with rRNA of the morphologically preserved cell, it is respectfully submitted that there simply cannot be any expectation of success in combining Kosse and Stender. This position is further supported by the Declaration of Dr. Stender[3] who states that Amann et al. would dissuade one of skill in the art from using enzyme-linked probes for the analysis of yeast. (**Fact 13**). Accordingly, it is respectfully submitted that the present rejection is improper and should be withdrawn.

It is well accepted that a "teaching away" is strong evidence of non-obviousness. *In re Braat,* 16 U.S.P.Q.2d 1529, 1533 (Fed. Cir. 1990) Appellants have argued in the prosecution history, and now reiterate for The Board, that Amann et al. teaches away from the presently claimed subject matter. Amann et al. state that they have never been able to achieve penetration of enzyme labeled probes into gram-positive bacteria or yeast cells (**Fact 5**). Amann et al. teach that the molecular weight of an enzyme-linked probe is approximately 50,000 and therefore many times larger than fluorescently labeled probes (**Fact 6**). It's size

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3 Dr. Stender has the proper credentials to form an expert opinion (See the Declaration attached as Appendix B; in particular see **Facts 10, 11 and 12**).

might be expected to hinder whole cell identification (**Facts 1, 6**). The analysis of several gram-positive bacteria and one yeast (*Saccharomyces cerevisiae*) were attempted by Amann et al. but could not be determined using enzyme-linked probes (**Facts 6 & 7**). Indeed, Amann et al. conclude from their study that enzyme-linked probes could only be successfully used for gram-negative bacteria and several members of Euryarchaeota (**Fact 8**). The clear implication of this reference is that despite their best efforts, Amann and his colleagues were still unable to use enzyme linked probes for the analysis of any gram-positive bacteria **or yeast cells**. This fact is further supported by the statement of Dr. Stender in his declaration wherein he states that to his knowledge, Dr. Amann and his colleagues have apparently never published a successful permeabilization method for yeast cells (**Fact 14**). In this respect the reference is a clear teaching away from the presently claimed subject matter.

Appellants would further submit that the Examiner's arguments regarding the combination of Kosse and Stender, when taken in view of Amann (the Examiner is reminded that the art must be considered as a whole and not piecemeal), is, at best, no more than an invitation to experiment or suggestion of what is obvious to try. This is clear because the combined references do not even provide general guidance as to the particular form of the claimed invention or how to achieve it.

For these reasons it is believed that each and every rejection under 35 U.S.C. § 103(a) must properly be withdrawn and all presently pending claims be allowed. In particular this applies to all claims being rejected based upon the arguments set forth in paragraphs 3, 4, 5 and 6 of the Office Action dated February 25, 2003. In particular this applies to the claims in Subgroups A & B, Groups I, II, III and IV.

c) Th Combination of References With D Wachter is not proper

De Wachter (GenBank Accession No. X58052) is nothing more than 18S

rRNA nucleotide sequence information. There are no associated teachings with that reference. Based upon this sequence disclosure the Examiner has opined that: "The nucleic acid of De Wachter is considered to have the property of being suitable for the detection, identification or quantitation of *Dekkera/Bretanomyces bruxellensis*." As noted above, it is the teachings of the references, and not the opinions of the Examiner, upon which a 35 U.S.C. §103(a) rejection must properly rest. More importantly, where the teachings of the art conflict with an Examiner's opinion, clearly the Examiner's opinion cannot be the basis for any rejection.

Kosse teaches that probes **must** be selected to yeast 18S rRNA regions that are fully accessible (**Fact 2**). Kosse also teaches that only the 3' end of 18S rRNA is accessible for species-specific probes (**Facts 21, 22 and 23**). In particular, Kosse teaches that only positions 1682-1728 are "accessible for species-specific probes" (**Fact 21**). By definition, probes for the detection, identification or quantitation of *Dekkera/Bretanomyces bruxellensis* would be species-specific probes. Accordingly, only very little of the sequence information of De Wachter could be considered to have the property of being suitable for the detection, identification or quantitation of *Dekkera/Bretanomyces bruxellensis*. Because the premise of the Examiner's argument is clear error, it is respectfully submitted that the asserted *prima facia* case for the combination of De Wachter with any of the foregoing references is improper and therefore must be withdrawn.

For these reasons it is believed that each and every rejection under 35 U.S.C. § 103(a) based upon a combination with De Wachter must properly be withdrawn. In particular this argument applies to all claims being rejected based upon the arguments set forth in paragraphs 5 and 6 of the Office Action dated February 25, 2003. In particular this applies to the claims in Subgroup B, Groups III and IV.

d) Certain Of Th Examiner's Arguments Are Not Well Tak n:

In the Advisory Action dated September 29, 2003, the Examiner states that Amann is not being used to reject the present claims. While this is true, the Examiner is reminded that the art must be considered as a whole and not piecemeal. Appellants have provided Amann and take the position that it is the most relevant art with respect to the application of enzyme linked probes for the *in-situ* analysis of yeast 18S rRNA because they appear to be the only persons to actually report the results of such an assay. The Examiner is reminded that Amann and his colleagues **failed to succeed**. Accordingly, with respect to any finding under 35 U.S.C. § 103(a), it is submitted that the Examiner should not attempt to cursorily dismiss the relevance of the Amann reference.

In her rebuttal arguments pertaining to the combination of Kosse and Stender (1998), the Examiner has argued with respect to Amann that: 1) there are no teachings in Amann which indicate that the enzyme linked probes cannot be applied to yeast; 2) Amann provides the results obtained when applying the enzyme-linked probes to the detection of S. cerevisiae; and 3) Amann does not teach that these results apply to the detection of all yeasts (**Fact 4**). Appellants would argue that all of the foregoing statements are misleading and in fact it is believed that the first statement is incorrect. More to the point however, is that the reference needs to be considered as a whole for what it teaches.

With regard to the first statement by the Examiner, it is respectfully submitted that Amann does state: "For now, enzyme-labeled oligonucleotides can therefore **be used only for** specific detection and identification of gram-negative bacteria and several members of *Euryarchaeota* (21)." (Emphasis added, **Fact 8**) This appears to Appellants as a pretty sound retort to the stated position of the Examiner.

With regard to the Examiner's second point, whilst it is correct that Amann provides the results obtained when applying the enzyme-linked probes to the detection of S. cerevisiae, the important point is that Amann **had no success** in

using enzyme-linked probes for such an analysis (**Facts 5, 7 & 8**). Consequently, when considered in view of the experiments with gram-positive bacteria and in the context of the discussions (and knowledge in the art) about how difficult it is to get a molecule with a large molecular weight through the cell wall of gram-positive bacteria and yeast (**Facts 1 & 6**), it is clear that when Amann is considered as a whole, the Examiner's omission causes her statement to be misleading.

With regard to her final statement, it is correct that Amann does not teach that these results apply to the detection of all yeasts. More importantly however, it is also correct that Amann teaches that they have never before (**Fact 5**), and still have not, been able to (**Fact 8**) use enzyme linked probes for the analysis of gram-positive bacteria or yeast. Moreover, Amann cites no literature where such assays have been successful. Accordingly, the ordinary practitioner is without any teaching whatsoever as to how to successfully perform such an assay. More to the point however, is that when such as assay was attempted by Amann and his colleagues, **it failed**.

The Examiner appears to rely substantially on the conclusory statement of Amann for her position (**Fact 9**) with respect to the combination of Kosse and Stender. It is respectfully submitted the conclusory paragraph of Amann amounts to wishful thinking and bald speculation. Most importantly, this statement of a panacea does not undermine the fact that Amann and his colleagues tried to used enzyme-linked probes for the analysis of gram-positive bacteria and yeast **and failed**. More importantly, since 1992, it appears that Amann and his colleagues have not been successful in the asserted endeavor (**Fact 14**) notwithstanding their **optimism** of 1992. Accordingly, little weight should be accorded given to the conclusory statements of Amann.

In the rebuttal arguments presented in the Office Action dated February 25, 2003, the Examiner states: "Thereby, Applicant's have not established that the probe of De Wachter labeled with an enzyme would not be suitable for in-situ

hybridization or other types of hybridization." With all due respect to the Examiner, it is a requirement under 35 U.S.C. § 103(a) that The Office establish a *prima facia* case based upon the teachings of the relevant art. Where a rejection is based upon an Examiner's assertions and the art relied upon by the Examiner does not support the premises of her assertions, it is improper to attempt to shift the burden of proof to Appellant to establish any facts whatsoever. Where the facts do not support a *prima facia* case for obviousness, any rejection is improper and properly must be withdrawn. Accordingly, the Examiner's attempt to shift any evidentiary proof burden to Applicant is simply an irrelevant distraction.

Summary

Because all arguments supporting the final rejection are clear error, it is respectfully requested that this honorable Board of Patent Appeals and Interferences reverse the Examiner's decision in this case and indicate the allowability of all pending claims.

In the event that this paper is not being timely filed, Appellant respectfully petitions for an appropriate extension of time. Any fees for such an extension together with any additional fees that may be due with respect to this paper may be charged to Counsel's Deposit Account 02-3240.

Respectfully submitted
On behalf of Applicants

February 18, 2004
Date:

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9. APPENDIX A

Claims on appeal

1. An enzyme-linked in-*situ* hybridization probe further characterized in that it comprises a probing nucleobase sequence that specifically hybridizes to a yeast specific target sequence.

2. The probe of claim 1, wherein the target sequence is ribosomal RNA.

3. The probe of claim 1, wherein the probe is a nucleic acid.

4. The probe of claim 1, wherein the probe is a peptide nucleic acid.

5. The probe of claim 1, wherein the probing nucleobase sequence is selected to detect, identify or enumerate organisms of one or more species of yeast.

6. The probe of claim 1, wherein the probing nucleobase sequence is selected to detect, identify or enumerate organisms of one or more genus of a yeast.

7. The probe of claim 1, wherein the probing nucleobase sequence is selected to detect, identify or enumerate all yeast in a sample.

8. The probe of claim 1, wherein the enzyme is selected from the group consisting of: a polymerase, alkaline phosphatase, horseradish peroxidase and soy bean peroxidase.

10. An enzyme-linked probe for detecting, identifying or quantitating the

presence of *Dekkera/Brettanomyces* yeast in a sample of interest, wherein the probe comprises a probing nucleobase sequence wherein at least a portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-GGT-GAG-GGT-CGC (Seq. ID No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA (Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.

11. The probe of claim 10, wherein the probing nucleobase sequence is selected to be one hundred percent homologous to a nucleobase sequence identified in the claim.

12. The probe of claim 10, wherein the probe is a peptide nucleic acid.

16. The probe of claim 10, wherein the probe is labeled with soy-bean peroxidase.

18. The probe of claim 10, wherein the probe is support bound.

19. The probe of claim 18, wherein the probe exists attached to an array of probes.

21. A set of enzyme-linked probes for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in a sample of interest, wherein one or more

of the probes comprise a probing nucleobase sequence wherein at least a portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-GGT-GAG-GGT-CGC (Seq. ID No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA (Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.

22. The probe set of claim 21, wherein the probing nucleobase sequences of said one or more probes are selected to be one hundred percent homologous to a nucleobase sequence identified in the claim.

23. The probe set of claim 21, wherein the probe set is specific for both the detection of *Dekkera/Brettanomyces* yeast as well as other organisms of interest in the same sample.

24. The probe set of claim 23, wherein the probes of the set are independently detectable.

25. The probe set of claim 21, wherein some of the probes of the set are blocking probes.

26. The probe set of claim 21, wherein all probes of the set are peptide nucleic acids.

29. The probe set of claim 21, wherein the probes are labeled with the enzyme soy-bean peroxidase.

32. The probe set of claim 21, wherein the probes are support bound.

34. A set of enzyme-linked probes for detecting, identifying or quantitating *Dekkera bruxellensis* yeast in a sample of interest, wherein the two or more probes specific for *Dekkera bruxellensis* yeast comprise a probing nucleobase sequence wherein at least portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5) and CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6) and sequences fully complementary thereto and of the same length.

46. A method for detecting, identifying or enumerating yeast in a sample of interest, said method comprising:

a) contacting one or more species of yeast in the sample with one or more yeast specific enzyme-linked probes, under suitable *in-situ* hybridization conditions, to thereby form one or more probe/target sequence hybrids within the yeast; and

b) detecting enzyme activity within the yeast to thereby determine the presence, absence or number of yeast sought to be detected in the sample.

47. The method of claim 46 further comprising the step of:

c) isolating the yeast using a filter as an isolation medium.

48. The method of claim 47, further comprising the step of:

d) growing the isolated yeast by culture in media.

49. The method of claim 48, wherein the culture is grown directly on the filter, under suitable culture conditions, by placing the filter in contact with media.

61. A method for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in a sample; said method comprising:

a) contacting one or more species of yeast in the sample with one or more *Dekkera/Brettanomyces* yeast specific probes, under suitable hybridization conditions, to thereby form a probe/target sequence hybrid; and

b) detecting the presence, absence or amount of probe/target sequence hybrid and correlating the result with the presence, absence or number of *Dekkera/Brettanomyces* yeast in the sample;

wherein one or more of the *Dekkera/Brettanomyces* yeast specific probes comprise a probing nucleobase sequence wherein at least a portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-GGT-GAG-GGT-CGC (Seq. ID No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA (Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.

62. The method of claim 61, wherein the probing nucleobase sequences of

said one or more probes are selected to be one hundred percent homologous to a nucleobase sequence identified in the claim.

80. A kit for performing an *in-situ* assay that detects, identifies or enumerates *Dekkera/Brettanomyces* yeast in a sample, wherein said kit comprises:
    a) a filter for isolating yeast from a sample of interest;
    b) culture media for growing the isolated yeast;
    c) fixation solution for fixing grown yeast;
    d) hybridization solution for imposing suitable *in-situ* hybridization conditions;
    e) an enzyme labeled probe specific for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in the sample; and
    f) one or more wash solutions for removing undesirable components after performing one or more steps of the assay.

81. The kit of claim 80, wherein the fixation solution and the hybridization solution are the same solution.

82. The kit of claim 80, wherein the soy bean peroxidase labeled probe is a peptide nucleic acid.

83. A method for quantitating slow growing yeast in a liquid sample in less than 48 hours; said method comprising:
    a) filtering a fixed volume of liquid using a filter having a pore size that does not allow the yeast to pass;
    b) incubating the filter containing the yeast, in media and under culture conditions, for 45 or fewer hours to thereby grow microcolonies of yeast;
    c) fixing the microcolonies of yeast to the filter;

d) contacting the microcolonies of yeast with a yeast specific enzyme-linked probe, under suitable *in-situ* hybridization conditions, to thereby form one or more probe/target sequence hybrids within the yeast;

e) detecting enzyme activity within the yeast to thereby determine the presence, absence or number of yeast sought to be detected in the sample; and

f) determining the quantity of yeast in the sample.

84. The method of claim 83, wherein fixing the microcolonies of yeast to the filter and contacting the microcolonies of yeast with a yeast specific enzyme-linked probe are performed simultaneously using a single solution.

85. The method of claim 83, wherein the number of CFU in the sample is determined.

86. The probe set of claim 10, wherein the enzyme is selected from the group consisting of: a polymerase, alkaline phosphatase, horseradish peroxidase and soy bean peroxidase.

87. The probe set of claim 21, wherein the enzyme is selected from the group consisting of: a polymerase, alkaline phosphatase, horseradish peroxidase and soy bean peroxidase.

Appendix B

BP9901-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Serial No:	**09/593,914** **Confirmati n No: 8319**
Date Filed:	June 14, 2000
Application Title:	Probes, Probe Sets, Methods And Kits Pertaining To The Detection, Identification And/Or Enumeration Of Yeast; Particularly In Wine
Applicants:	Hyldig-Nielsen et al.
Group Art Unit:	1634
Examiner:	C. Myers
Action Date:	February 25, 2003
Action Type:	Third Office Action On Merits - FINAL
Certified Mail No.:	7003 0500 0000 1731 7079

Certificate of Mailing Pursuant to:
37 C.F.R. § 1.8

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Mail Stop: AF, Commissioner For Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on this 22nd day of August, 2003.

Brian D. Gildea
Reg. No. 39,995

DECLARATION OF DR. HENRIK STENDER
UNDER 37 C.F.R. § 1.132

Commissioner for Patents
Dear Sir or Madam:

I, Dr. Henrik Stender of Fasanhaven 5, DK-2820 Gentofte, Denmark do hereby declare and state that:

1. I am presently employed as Vice President of Research & Development of AdvanDx, Inc. and have been in this position since August 2002;

Appendix B

2. I was formerly employed at Boston Probes & Applied Biosystems from May, 1998 until August, 2002 in various positions, including Director of Microbiology;

3. Before that I was employed at Dako A/S Denmark as Research Scientist from July, 1992 until May, 1998;

4. I received my doctorate from Technical University of Denmark in 1992 in the area of Immunology;

5. I have been employed as a scientist/manager in the field of assay development for a total of 11 years;

6. I am a co-inventor of the above captioned patent application and I have reviewed the claims as currently pending in the application;

7. I have reviewed the Office Action dated February 25, 2003 and the examiners arguments set forth therein;

8. I am a co-inventor of PCT/DK97/00425 (WO98/15648) entitled: "Novel Probes For The Detection Of Mycobacteria", referred to by the Examiner in the above captioned application as Stender (1998) and am familiar with its contents;

9. I have reviewed Kosse et al., Systems. Appl. Microbiol. 20: 468-480 (1997);

10. I have reviewed Amann et al., Applied and Environmental Microbiology 58(9): 3007-3011 (1992);

11. Based upon my review of the Amann et al. reference, I believe that: 1) Amann et al. teach that it was well accepted, at the time of their publication, that enzyme-linked (labeled) probes COULD NOT readily penetrate the cell wall of yeast; 2) Amann et al. had no success with getting enzyme-labeled probes into yeast; and 3) Amann et

al. would tend to dissuade one of skill in the art from attempting to use an enzyme-linked probe to analysis a yeast in an in-situ based assay;

12. Kosse et al. would not, in view of Amann et al., tend to motivate the application of enzyme-linked probes to in-situ assays for yeasts because the reference does not address this particular assay format;

13. Stender (1998) would not, in view of Amann et al. tend to motive the application of enzyme-linked probes to in-situ assays for yeasts because the reference does not address yeasts;

14. One of skill in the art at the time of the present invention would not have a reasonable expectation of successfully applying enzyme-linked probes to the determination of yeast in an in-situ based assay because there was inadequate teaching available as to how to permeablize the cell wall of the yeast to these large molecules.

I further declare that all statements made in this Declaration are true and that all statements made on information and belief are believed to be true. Moreover, these statements were made with the knowledge that willful false statements and the like made by me are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Henrik Stender

8/21 - 2003

Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

APPEAL BRIEF FOR APPELLANTS

Jens J. Hyldig-Nielsen
Heather P. O'Keefe
Henrik Stender

Probes, Probe Sets, Methods And Kits Pertaining To The Detection, Identification And/or Enumeration Of Yeast; Particularly in Wine

Serial No. 09/593,914
Filed: June 14, 2000
Group Art Unit: 1634
Appeal No.:

The attached Appeal Brief is submitted in accordance with the notice mailed on August 22, 2003. A petition under 37 C.F.R. § 1.136 for an automatic 4-month extension of time is being filed herewith thereby making this Appeal Brief due on February 22, 2004. All other appropriate fees for the Appeal Brief have been paid. A request for an Oral Hearing, and payment of the appropriate fee accompanies this Appeal Brief. No other fees are believed to be due for the filing of this paper but if The Office disagrees, The Office is authorized to deduct the appropriate fee from Deposit Account 02-3240.

Respectfully submitted,

February 18, 2004
Date:

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Appeal Brief (in triplicate)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re the application of: Appeal No.

Hyldig-Nielsen et al. Examiner: Carla Myers

Serial Number: 09/593,914 Group Art Unit: 1634

Filed: June 14, 2000

For: Probes, Probe Sets, Methods And Kits Pertaining To The Detection, Identification And/Or Enumeration Of Yeast; Particularly In Wine

BRIEF ON APPEAL

February 18, 2004

INTRODUCTION

This is an appeal from the action of the Examiner dated February 25, 2003, finally rejecting claims 1-8, 10-12, 16, 18, 19, 21-26, 29, 32, 24, 46-49, 61, 62 and 80-87, all of the claims pending in this application. The Examiner has maintained a restriction requirement with respect to claims 10, 11, 21, 22, 34, 61 and 62. Appellants did traverse the restriction requirement, did provide appropriate and timely filed arguments in response to the restriction requirement as well as a timely filed petition under 37 C.F.R. § 1.144 on August 22, 2003 requesting the withdrawal of the restriction requirement.

In addition, claims 1-8 and 46 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Kosse (reference DF) in view of Stender (1998; WO98/15648; reference BB). Claims 47-49 and 80-85 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Kosse (reference DF) in view of Stender (1998; WO98/15648; reference BB) and in further view of Parton (US 5,905,038). Claims 1-8, 10-12, 16, 18-19, 21-26, 29, 32, 33, 46, 61-62, 86 and

87 stand rejected under 35 U.S.C. §103(a) as being unpatentable over De Wachter (GeneBank Accession No. x58052) in view of Kosse (reference DF) in further view of Stender (1998; WO98/15648; reference BB). Claims 47-49 and 80-85 stand rejected under 35 U.S.C. §103(a) as being unpatentable over De Wachter in view of Kosse (reference DF) and Stender (1998; WO98/15648; reference BB) in further view of Parton (US 5,905,038). A Notice of Appeal was timely filed on August 22, 2003. A petition under 37 C.F.R. § 1.136(a) for an automatic four-month extension of time is being filed with this brief. Accordingly, it is believed that this Brief is due on or before February 22, 2004 and is therefore being timely filed. Accordingly, please consider this Brief On Appeal.

1. REAL PARTY IN INTEREST

The application has been assigned to Boston Probes, Inc., 15 DeAngelo Drive, Bedford, MA. USA. As of the filing of this Brief On Appeal, Boston Probes, Inc. is wholly owned by Applera Corporation, acting through its Applied Biosystems stock group. These stand as the parties having an interest herein.

2. STATEMENT OF RELATED APPEALS AND INTERFERENCES

No related appeals and/or interferences are pending.

3. STATUS OF CLAIMS

Claims 1-8 and 46 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Kosse (reference DF) in view of Stender (1998; WO98/15648; reference BB). Claims 47-49 and 80-85 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Kosse (reference DF) in view of Stender (1998; WO98/15648; reference BB) and in further view of Parton (US 5,905,038). Claims 1-8, 10-12, 16, 18-19, 21-26, 29, 32, 33, 46, 61-62, 86 and 87 stand rejected under 35 U.S.C. §103(a) as being unpatentable over De Wachter (GeneBank Accession No. x58052) in view of Kosse (reference DF) in further

view of Stender (1998; WO98/15648; reference BB). Claims 47-49 and 80-85 stand rejected under 35 U.S.C. §103(a) as being unpatentable over De Wachter in view of Kosse (reference DF) and Stender (1998; WO98/15648; reference BB) in further view of Parton (US 5,905,038). No claim stands allowed.

4. STATUS OF AMENDMENTS

All amendments of record appear to have been entered. The claims set forth in Section 9 (Appendix) reflect the entry of all amendments. No new amendments are offered.

5. SUMMARY OF THE INVENTION

The present invention pertains to probes, probe sets, methods and kits for the detection, identification and/or enumeration of yeast; particularly in wine (Title).

Accordingly, in some embodiments, the invention pertains to enzyme-linked probes suitable for use in an *in-situ* hybridization assay wherein the probes comprise a probing nucleobase sequence directed to a target sequence within yeast. (Specification at page 15, lines 26-28). In some embodiments, the enzyme-linked probe can be suitable for detecting, identifying or quantitating *Dekkera/Brettanomyces*, and particularly *Dekkera bruxellensis* yeast, in a sample of interest. (Specification at page 16, lines 12-14). Preferred probing nucleobase sequences of said yeast specific probes are listed in Table 1. (Specification at page 16, lines 16-17)

In some embodiments, the invention pertains to probe sets suitable for detecting, identifying or quantitating *Dekkera/Brettanomyces in-situ*, and particularly *Dekkera bruxellensis* yeast, in a sample of interest. (Specification at page 17, lines 20-22) Preferred probing nucleobase sequences of said yeast specific probes are listed in Table 1. (Specification at page 17, lines 22-24)

In some embodiments, the invention pertains to a method for detection,

identification or quantitation of yeast using enzyme-linked probes in an *in-situ* hybridization (ISH) assay. (Specification at page 19, lines 32-34). The method comprises contacting one or more species of yeast with a yeast specific enzyme-linked probe, under suitable *in-situ* hybridization conditions, to thereby form a probe/target sequence hybrid. (Specification at page 19-20, bridging sentence). The enzyme activity can then be used to detect, identify or quantitate yeast present in the sample. (Specification at page 19-20, bridging sentence). In some embodiments, the method is applied to the determination of *Dekkera/Brettanomyces* yeast. (Specification at page 20, lines 10-27)

In some embodiments, the invention pertains to kits for performing an *in-situ* assay that detects, identifies or enumerates *Dekkera/Brettanomyces*, and particularly *Dekkera bruxellensis* yeast, in a sample of interest. (Specification at page 23, lines 30 to page 31, line 28)

In some embodiments, the invention pertains to methods, kits and compositions that are particularly useful for the rapid *in-situ* probe based detection, identification and quantitation of slow growing yeast. (Specification at page 22, lines 11-13). Said assays can typically be completed within 27-48 hours. (Specification at page 23, lines 24-25).

6. ISSUES ON APPEAL

Issue 1:

The Examiner issued a restriction requirement in the Office Action dated July 18, 2001. In a response dated January 17, 2002, Appellants responded to the restriction requirement by making an election as required by the rules of practice and submitting arguments supporting a traverse of the restriction requirement. Appellants submitted a timely petition under 37 C.F.R. § 1.144 on August 22, 2003 requesting withdrawal of the restriction requirement and reiterating the arguments of record. Appellants have not been informed as to the

outcome of said petition. Assuming that the restriction requirement is not withdrawn as a result of the timely filed petition, Appellants believe the restriction requirement is improper and therefore seek withdrawal of the restriction requirement by The Board. For brevity, The Board is requested to review the arguments set forth in the petition under 37 C.F.R. 1.144 file on August 22, 2003.

Issue 2:

Claims 1-8, 10-12, 16, 18, 19, 21-26, 29, 32, 34, 46-49, 61, 62, 80-87 stand pending in the application. All pending claims have been rejected under 35 U.S.C. § 103(a) in view of various combinations of references. No claim stands allowed.

All rejections under 35 U.S.C. § 103(a) are respectfully traversed. The issue on Appeal is whether or not each of these pending rejections is proper. Appellants request that The Board overrule the Examiner and withdraw the rejections.

7. GROUPING OF CLAIMS

Because there are four distinct rejections under 35 U.S.C. § 103(a) that remain pending, it is believed that at least four claim groups (Groups I-IV) exist. It is believed that within certain of the groups, there are subgroups that exist based upon specific arguments set forth below. Accordingly it is believed that the grouping and subgrouping of the claims is as follows:

Group I, Claims 1-8 and 46 based upon the combination of Kosse (reference DF) with Stender (1998; WO98/15648; reference BB);

Group II, Claims 47-49 and 80-85 based upon the combination of Kosse (reference DF) with Stender (1998; WO98/15648; reference BB) in further view of Parton (US 5,905,038);

Group III, Claims 1-8, 10-12, 16, 18-19, 21-26, 29, 32, 46, 61-62, 86 and 87 based upon the combination of De Wachter (GeneBank Accession No. ----

x58052) with Kosse (reference DF) in further view of Stender (1998; WO98/15648; reference BB);

Group IV, Claims 47-49 and 80-85 based upon the combination of De Wachter (GeneBank Accession No. x58052) with Kosse (reference DF) and Stender (1998; WO98/15648; reference BB) in further view of Parton (US 5,905,038).

Subgroups **B** and **A** include those rejections based upon the De Wachter reference and those that do not, respectively. Thus, Subgroup A includes Groups I and II and Subgroup B includes Groups III and IV.

8. APPELLANT'S ARGUMENTS

I. Statement Of The Facts

Appellants reproduce below, for the convenience of the Board, such facts and evidence as support certain of the forthcoming arguments. Appellants note that facts and evidence are a product of the record. The record includes the specification as filed as well as each of the references considered during prosecution.[1] The source for each fact is noted with emphasized text indicated in bold, as appropriate.

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1 See: *In re Wiseman*, 596 F.2d 1019, 1023, 201 U.S.P.Q. 658, 661 (C.C.P.A. 1979) where the court looks to the specification for support regarding Appellant's claim to unexpected results. Moreover, the court in *In re Schulze* looked to the specification for support of a claim to unexpected results by stating: "Nor do we find anything in the record by way of **disclosure** (emphasis added) or affidavit ..." (*In re Schulze*, 52, C.C.P.A. 1422, 1424, 346 F.2d 600, 602, 145 U.S.P.Q. 716, 718 (C.C.P.A. 1965) Furthermore, "In determining whether the invention as a whole would have been obvious under 35 U.S.C. § 103, we must first delineate the invention as a whole. In delineating the invention as a whole, we look not only to the subject matter which is literally recited in the claim in question (the ratio of values) but also to those properties of the subject matter which are inherent in the subject matter and are **disclosed in the specification** (emphasis added)." *In re Antonie*, 559 F.2d 618, 619, 195 U.S.P.Q. 6, 8 (Fed. Cir. 1977). Finally, what a reference teaches is a **question of fact**. *In re Bell*, 991 F.2d 781, 784, 26 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1993).

A. FACTS ESTABLISHED BY THE SPECIFICATION

Fact 1:

"However, the use of enzyme-labeled DNA probes for the detection of yeast cells by *in-situ* hybridization has not yet been demonstrated (Amann, R. I., Zarda, B., Stahl, D.A. and Schleifer, K.-H., **Identification of individual prokaryotic cells by using enzyme-labeled, rRNA-targeted oligonucleotide probes**, *Applied and Environmental Microbiology, 58:* 3007-3011 (1992)) and Applicants are unaware of any attempts to use enzyme-labeled PNA probes to detect yeast by *in-situ* hybridization. The lack of examples of successful ISH assays utilizing enzyme linked probes likely results because of difficulties in getting such large molecules to pass through the cell membrane into the yeast cytoplasm." (Emphasis original; Specification at pages 3-4 bridging paragraph).

B. ADMISSIONS/STATEMENTS BY THE EXAMINER

Fact 2:

"The reference (Kosse) teaches that prior to *in situ* hybridization, yeast cell walls **must** be permeabilized and that probes **must** be selected to yeast 18S rRNA regions which are fully accessible to probes (see page 478)." (Emphasis and annotations added; Office Action dated February 25, 2003 at page 3)

Fact 3:

"Kosse teaches that the *in situ* hybridization method is performed using fluorescent-labeled probes and that dot blot hybridization is performed using digoxigenin labeled probes. Kosse **does not** specifically teach using enzyme-linked probes, or specifically soy bean peroxidase labeled probes." (Emphasis added; Office Action dated February 25, 2003 at page 3)

Fact 4:

The Examiner has argued: "However, as stated in the office action of Paper

No. 13, there are no teachings in Amann which indicate that the enzyme linked probes cannot be applied to the detection of yeasts. Amann provides the results obtained when applying the enzyme-linked probes to the detection of S. cerevisiae. However, Amann does not teach that these results apply to the detection of all yeasts" (Office Action dated February 25, 2003 at page 4)

C. FACTS ESTABLISHED BY AMANN ET AL., APPLIED AND ENVIRONMENTAL MICROBIOLOGY, 58(9): 3007-3011 (1992);

REFERENCE CA

Fact 5:

"Hitherto we had not achieved penetration of enzyme-labeled probe into gram positive bacteria **or yeast cells**." (Emphasis added; Amann et al., at the Abstract).

Fact 6:

"The molecular weight of horseradish peroxidase (40,000) is approximately 100 times greater than that of fluorescein or tetramethylrhodamine, **the two most common labels of rRNA-targeted oligonucleotide probes for single-cell identification. This increases the overall molecular weight of a probe molecule from approximately 6,000 to about 50,000, and penetration of enzyme-labeled probe through the cell periphery might be expected to hinder whole-cell identification**. However, we were encouraged by our previous studies demonstrating detection of DIG-labeled oligonucleotides with HRP-labeled anti-DIG Fab fragments (22). These conjugates have a molecular weight of at least 100,000, but nevertheless probe-conferred, HRP-mediated intracellular formation of a substrate precipitate could be demonstrated for gram-negative cells after lysozyme-EDTA treatment. Thus, it was not surprising that an enzyme-probe conjugate could also penetrate all strains of gram-negative bacteria examined in this study. But again this was effective only after lysozyme-

EDTA treatment. **On the other hand, th gram-positive bacteria xamined (*B. subtilis* and several strains of lactococci) remained impermeable to HRP-labeled probe even after prolonged incubation with lysozyme, lysostaphin (Sigma), or mutanolysin (Sigma) at different concentrations.** Following extensive digestion, the gram positive bacteria were only partly strained while gram negative bacteria, added as a control, were lysed (data not shown). Proteinase K (Boehringer Mannheim) treatment was effective but could not be controlled in a way to preserve morphological integrity of the cells. They could hardly be visualized by phase-constant microscopy and hybridized weakly probably because of loss of target molecules (data not shown)." (Emphasis added; Amann et al., at page 3008-3010, bridging paragraph).

Fact 7:

"We encountered similar problems with cells of *Saccharomyces cerevisiae*, and again attempts to make the intracellular RNA accessible by enzymatic pretreatment (lyticase and β–glucoronidase; both from Sigma) of the cells or detergent addition **failed**." (Emphasis added; Amann et al., at page 3010, col. 1).

Fact 8:

"**For now, enzyme-labeled oligonucleotides can therefore be used only for specific detection and identification of gram-negative bacteria and several members of *Euryarchaeota* (21)**." (Emphasis added; Amann et al. at page 3010, col. 1).

Fact 9:

"We are continuing to evaluate alternative approaches to permeabilize whole fixed cells. **Although we are optimistic** that methods can be tailored for single strains under investigation, we do not expect to find a universal method that will permeabilize the whole array of different microorganisms to a

comparable degree. This means that general probes (e.g., for the three domains) should not be used as enzyme derivatives for the characterization of environmental samples because they will likely produce biased results. Nevertheless, highly specific enzyme-probe conjugates used in combination with suitable methods to permeabilize specific cells of interest should provide a valuable tool for the detection and identification of individual cells *in situ*. (Emphasis added; Amann et al. at page 3010, col. 1 to col. 2, bridging paragraph).

D. FACTS ESTABLISHED BY THE DECLARATION OF DR. HENRIK STENDER[2]

In his Declaration of August 21, 2003 (Appendix B), Dr. Stender made the following statements now entered as Facts:

Fact 10:

"I am a co-inventor of PCT/DK97/00425 (WO98/15648) entitled: "Novel Probes For The Detection Of Mycobacteria", (herein either Stender (1998; WO98/15648; reference BB) or Stender (1998)) referred to by the Examiner in the above captioned application (09/593,914) as Stender (1998) and am familiar with its contents" (Annotation added; Stender Declaration at page 2)

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2 In the Advisory Action dated September 29, 2003, the Examiner acknowledges that she refused to consider the Declaration of Dr. Stender stating that it raises new issues and was not timely filed. With all due respect, the issue of what Amann and his colleagues teach has been raised by Appellants from as far back as the Specification (**Fact 1**). What Amann and the other references teach is a matter of fact and has been repeatedly treated in the prosecution history of this application. Accordingly, the statement that the Declaration of Dr. Stender raises new issues appears to be evasive and inaccurate. Accordingly, Appellants believe the Declaration of Dr. Stender was properly filed and should have been considered because the Examiner has continuously misinterpreted the actual teachings of the references as well as their impact on the impressions of the ordinary practitioner with respect to the requirements of an obviousness determination under 35 U.S.C. § 103(a).

Fact 11:

"I have reviewed Kosse et al., Systems. Appl. Microbiol. 20: 468-480 (1997)" (Stender Declaration at page 2)

Fact 12:

I have reviewed Amann et al., Applied and Environmental Microbiology 58(9): 3007-3011 (1992)" (Stender Declaration at page 2)

Fact 13:

"Based upon my review of the Amann et al. reference, I believe that: 1) Amann et al. teach that it was well accepted, at the time of their publication, that enzyme-linked (labeled) probes COULD NOT readily penetrate the cell wall of yeast; 2) Amann et al. had no success with getting enzyme-labeled probes into yeast; and 3) Amann et al. would tend to dissuade one of skill in the art from attempting to use an enzyme-linked probe to analysis [sic] a yeast in an in-situ based assay" (Annotation added; Stender Declaration at page 2-3, bridging paragraph)

Fact 14:

"Amann et al. has, to my knowledge, never published a successful permeabilization method for yeasts for in situ analysis with enzyme-labeled probes" (Stender Declaration at page 3)

Fact 15:

"Kosse et al. would not, in view of Amann et al., tend to motivate the application of enzyme-linked probes to in-situ assays for yeasts because the reference does not address this particular assay format" (Stender Declaration at page 3)

Fact 16:

"Stender (1998) would not, in view of Amann et al. tend to motive the application of enzyme-linked probes to in-situ assays for yeasts because the reference does not address yeasts" (Stender Declaration at page 3)

Fact 17:

"One of skill in the art at the time of the present invention would not have a reasonable expectation of successfully applying enzyme-linked probes to the determination of yeast in an in-situ based assay because there was inadequate teaching available as to how to permeablize the cell wall of the yeast to these large molecules." (Stender Declaration at page 3)

E. FACTS ESTABLISHED BY KOSSE ET AL., SYSTEM. APPL. MICROBIOL. 20: 468-480 (1997)

Fact 18:

"Therefore, the applicability of whole cell hybridization with **fluorescently labeled oligonucleotide probes** for the identification of eucaryotic yeasts from dairy products was investigated." (Emphasis added; Kosse et al. at page 469, col. 1)

Fact 19:

"The first step for hybridization of whole yeast cells to oligonucleotide probes is the **permeabilization** of the cell wall which might prevent penetration of the probes." (Emphasis added; Kosse et al. at page 474 col. 1)

Fact 20:

"Prior to in situ hybridization, yeast cell walls **must be permeabilized**." (Emphasis added; Kosse et al. at page 478, col. 1)

Fact 21:

"Experiments with a variety of species-specific fluorescently labeled oligonucleotides revealed that **only the 3'-end of 18S rRNA is accessible for species specific probes**." (Emphasis added; Kosse et al. at page 474, col. 1) It is noted that Figure 3 of Kosse et al. indicates that only positions 1682-1728 are "accessible for species-specific probes".

Fact 22:

"Therefore, we take our results as evidence that the target regions of these probes on the yeast 18S rRNA **were not fully accessible**." (Emphasis added; Kosse et al. at page 478, col. 2)

Fact 23:

"The **restricted accessibility** of potential target sites on 18S rRNA for species-specific probes currently limits the applicability of fluorescence-*in situ*-hybridization for yeasts. In these cases, alternative hybridization techniques based upon the extraction of nucleic acids must be used, e.g., the dot blot technique with DIG-labeled probes." (Emphasis added; Kosse et al. at page 478, col. 2)

F. FACTS ESTABLISHED BY STENDER (1998; WO98/15648; REFERENCE BB)

Appellants have presented the following as Facts at page 8-9 of the Office Action response dated December 6, 2002. Because the Examiner has apparently not taken issue with any of the foregoing, it is believed that no dispute exists as to these facts.

Fact 24:

"Stender (1998) does not teach anything about yeasts but is limited to determinations of mycobacteria."

Fact 25:

"Stender (1998) teaches enzyme-linked probes for the determination of mycobacteria but does not teach about permeabilizing yeast cells to enzyme-linked probes."

II. The Law Of 35 U.S.C. § 103(a)

1. General Standard:

"A claimed invention is unpatentable if the differences between it and the prior art "are such that the subject matter **as a whole** (emphasis added) would have been obvious at the time the invention was made to a person of ordinary skill in the art." *In re Dembiczak*, 175 F.3d 994, 998, 50 U.S.P.Q.2d 1614, 1616 (Fed. Cir. 1999). In determining whether a claimed invention is obvious one must consider; 1) the scope and content of the prior art; 2) the level of skill in the prior art; 3) the differences between the claimed invention and the prior art; and 4) objective evidence of non-obviousness such as secondary factors. Id.

The PTO bears the burden under 35 USC § 103 to establish an unrebutted *prima facia* case of obviousness. *In re Rouffet*, 149 F.3d 1350, 1355, 47 U.S.P.Q.2d 1453, ___ (Fed. Cir. 1998). To satisfy its burden, the PTO must show some objective teaching in the prior art or that knowledge generally available in the art would lead the ordinary practitioner to combine **relevant teaching**. *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598 (Fed. Cir. 1988). In the absence of a proper *prima facia* case of obviousness, an Applicant is entitled to a patent. *In re Rouffet*, 149 F.3d 1350, 1355, 47 U.S.P.Q.2d 1453, ___ (Fed. Cir. 1998). To overcome a claimed *prima facia* case of obviousness, an Applicant can either show that the *prima facia* case of obviousness is

insufficient because it relies on incorrect factual predicates or otherwise present secondary evidence of non-obviousness. *Id.*

A proper rejection under 35 USC § 103 may not be premised upon "bald assertions" for which there is no support for or explanation of a conclusion. *Id.* An Examiner's cursory statement unaccompanied by evidence or reasoning is entirely inadequate to support a rejection. *In re Sichert*, 566 F.2d 1154, 1164, 196 U.S.P.Q. 209, 217 (C.C.P.A., 1977). A rejection based on section 103 must be based in fact that is not aided by hindsight. *In re Warner*, 54 C.C.P.A. 1628, 1635, 379 F.2d 1011, 1017, 154 U.S.P.Q. 173, 178 (C.C.P.A., 1967). The PTO may not resort to speculation, unfounded assumptions or hindsight reconstruction to supply deficiencies in its factual basis for a rejection. *Id.* Doubts as the factual basis for a rejection must to resolved in favor of the Applicant since it is the PTO's burden to establish a *prima facia* case of obviousness. *Id.*

2. Application of Hindsight:

On the permissibility of an Examiner's use of hindsight, the law is quite clear. Recent courts have held that: "Our analysis begins in the text of section 103 quoted above, with the phrase "at the time the invention was made". For it is this phrase that guards against the "tempting but forbidden zone of hindsight,"." *In re Dembiczak*, 175 F.3d 994, 998, 50 U.S.P.Q.2d 1614, 1617 (Fed. Cir. 1999). Measuring the claimed invention against the standard established by section 103 requires the oft-difficult but critical step of casting the mind back to the time of invention, to consider the thinking of one of ordinary skill in the art, guided only be the prior art references and **the then-accepted wisdom in the field** (emphasis added)." *Id.* Close adherence to this methodology is especially important to avoid using the inventor's own teachings against the teacher. *Id.* The best defense against application of hindsight is **rigorous application** of a requirement for a showing of the teaching or motivation to combine the prior art references. *Id.* The examiner can satisfy this burden only by showing some objective teaching

leading to the combination. *Id.* That showing must be **clear and particular**. *Id.* "Broad conclusory statements regarding the teaching of multiple references, standing alone, are not "evidence." *Id.* The mere fact that the prior art may be modified in a manner suggested by the Examiner does not make the modification obvious unless the prior art expressly suggested the modification. *In re Fritch*, 972 F.2d 1260, 1266, 23 U.S.P.Q.2d 1780, 1783-1784 (Fed. Cir. 1992). Moreover, when prior art references require selective combination, there must be some reason for the combination other than the hindsight gleaned from the invention itself. *Interconnect Planning Corp. v. Feil*, 774 F.2d. 1132, 1143, 227 U.S.P.Q. 543, 551 (Fed. Cir. 1985). The Examiner may not pick and choose among isolated disclosures to deprecate the claimed invention. *In re Fritch*, 972 F.2d 1260, 1266, 23, U.S.P.Q.2d 1784, 1784 (Fed. Cir. 1992).

3. Obvious To Try Is Not The Standard Of Obviousness

"Obvious to try" is not a legitimate test of patentablility. *In re Fine,* at 1075, 5 U.S.P.Q.2d 1596,1599 (Fed. Cir. 1988). Admonition that the subject invention is "obvious to try" is generally directed to two types of error. In one case (motif one), that which would be "obvious to try" is to vary all parameters or to try each of numerous possible choices until one arrives at a successful result, where the prior art gave no indication of which parameters where critical and no directions as to which of many possible choices were likely to be successful. In the other case (motif two), that which is "obvious to try" is to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it. *In re O'Farrell*, 853 F.2d 894, 903, 57 USLW 2147, ___, 7 U.S.P.Q.2d 1673, 1681 (Fed. Cir. 1988).

4. Expectations of Success

Both suggestion and reasonable expectation of success must be found in

the prior art *and not in Applicant's disclosure*. *In re Vaeck*, 947 F.2d 448, 493, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991). Moreover, a prior art disclosure should be considered as much for how the teachings differ with the subject invention as well as how they are similar. *Id.* at 494, 20 U.S.P.Q.2d 1438, 1443.

III. Arguments Supporting Patentability

a) All Of The Present Rejections Are Prima Facia Deficient

As noted above, the burden rests with The Office, and specifically the Examiner, to, at a minimum, establish a *prima facia* case for obviousness else the Applicants (herein the Appellants) are entitled to a patent. To establish a *prima facia* case for obviousness the Examiner state the **motivation** to combine relevant art. The mere fact that the prior art may be modified in a manner suggested by the Examiner does not make the modification obvious unless the prior art expressly suggested the modification. *In re Fritch*, 972 F.2d 1260, 1266, 23 U.S.P.Q.2d 1780, 1783-1784 (Fed. Cir. 1992).

It is respectfully submitted that upon careful review of each of the rejections appearing at paragraphs 3, 4, 5, or 6 of the Office Action dated February 25, 2003, it is clear that the Examiner has failed to describe any particular motivation to combine the references. In each case, the Examiner simply asserted that it was *prima facia* obvious to combine the references to thereby achieve the presently claimed subject matter. Such a rejection merely takes elements from various references and combines them using the Appellant's specification as a blueprint to achieve a description of the claimed subject matter. Such a rejection is hindsight based and clearly prohibited by precedent such as *In re Dembiczak*, 175 F.3d 994, 50 U.S.P.Q.2d 1614 (Fed. Cir. 1999).

For these reasons it is believed that each and every rejection under 35 U.S.C. § 103(a) must properly be withdrawn and all presently pending claims be allowed. In particular this applies to all claims being rejected based upon the arguments set forth in paragraphs 3, 4, 5 and 6 of the Office Action dated

February 25, 2003. In particular this applies to the claims in Subgroups A & B, Groups I, II, III and IV.

b) The combination of Kosse with Stender is Improper

Each and every one of the presently pending rejections requires the proper combination of Kosse (reference DF) with Stender (1998; WO98/15648; reference BB). In addition to lacking a proper motivation to combine the references, it is respectfully submitted that the Examiner has committed the following additional errors:

1) The art is non-analogous;
2) There is no reasonable expectation of success;
3) The art teaches away from the claimed invention;
4) Obvious to try is not the standard under 35 U.S.C. § 103(a).

It is well accepted that any rejection based upon 35 U.S.C. §103(a) must rely upon analogous art. M.P.E.P. § 2141.01(a). It is respectfully submitted that Stender (1998; WO98/15648; reference BB) is non-analogous art.

All of the presently pending claims pertain to the methods, kits and composition suitable for the in-situ detection, identification and/or enumeration of **yeast**. Whilst Kosse (reference DF) pertains to the determination of yeast with fluorescently labeled probes (**Fact 3, 15, 18**), Stender (1998; WO98/15648; reference BB) pertains to the determination of mycobacteria, not yeast (**Facts 10, 16, 24 & 25**). It is well established that to perform an *in-situ* assay on yeast, the cell walls of the yeast must be permeable to the probe (**Facts 1, 2, 6, 17, 19 & 20**) else no determination can be made. There is no evidence presented by the Examiner that the references in any way teach that mycobacteria are in any way related to yeast so that permeabilization techniques applied to mycobacteria can be, or might be, successfully applied to yeast. Many Facts suggest that no such conclusion can be drawn (e.g. **Facts 7, 8, 13, 14, 16 and 17**). Accordingly it is believed that Stender is non-analogous art and for at least this reason, it is not

properly combined with Kosse.

It is well accepted that in addition to motivation to combine references, the references themselves must provide a reasonable expectation of success in the practice of the elements as combined. That expectation must be clear from the teaching of the references and not opined by the Examiner. Appellants reiterate that Stender (1998) pertains to the determination of mycobacteria, not yeast (**Facts 10, 16, 24 & 25**). Kosse pertains to the determination of yeast with fluorescently labeled probes (**Fact 3, 15, 18**) and by the Examiner's own statements, Kosse doe not teach, disclose or even suggest the use of enzyme-linked probes (**Fact 3**) or methods for the permeabilization of cell wall of yeast to these large molecules. Because there is not a single reference that teaches the ordinary practitioner about how to permeabilize the cell walls of a yeast so as to enable an enzyme-linked probe to effectively interact with rRNA of the morphologically preserved cell, it is respectfully submitted that there simply cannot be any expectation of success in combining Kosse and Stender. This position is further supported by the Declaration of Dr. Stender[3] who states that Amann et al. would dissuade one of skill in the art from using enzyme-linked probes for the analysis of yeast. (**Fact 13**). Accordingly, it is respectfully submitted that the present rejection is improper and should be withdrawn.

It is well accepted that a "teaching away" is strong evidence of non-obviousness. *In re Braat,* 16 U.S.P.Q.2d 1529, 1533 (Fed. Cir. 1990) Appellants have argued in the prosecution history, and now reiterate for The Board, that Amann et al. teaches away from the presently claimed subject matter. Amann et al. state that they have never been able to achieve penetration of enzyme labeled probes into gram-positive bacteria or yeast cells (**Fact 5**). Amann et al. teach that the molecular weight of an enzyme-linked probe is approximately 50,000 and therefore many times larger than fluorescently labeled probes (**Fact 6**). It's size

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3 Dr. Stender has the proper credentials to form an expert opinion (See the Declaration attached as Appendix B; in particular see **Facts 10, 11 and 12**).

might be expected to hinder whole cell identification (**Facts 1, 6**). The analysis of several gram-positive bacteria and one yeast (*Saccharomyces cerevisiae*) were attempted by Amann et al. but could not be determined using enzyme-linked probes (**Facts 6 & 7**). Indeed, Amann et al. conclude from their study that enzyme-linked probes could only be successfully used for gram-negative bacteria and several members of Euryarchaeota (**Fact 8**). The clear implication of this reference is that despite their best efforts, Amann and his colleagues were still unable to use enzyme linked probes for the analysis of any gram-positive bacteria **or yeast cells**. This fact is further supported by the statement of Dr. Stender in his declaration wherein he states that to his knowledge, Dr. Amann and his colleagues have apparently never published a successful permeabilization method for yeast cells (**Fact 14**). In this respect the reference is a clear teaching away from the presently claimed subject matter.

Appellants would further submit that the Examiner's arguments regarding the combination of Kosse and Stender, when taken in view of Amann (the Examiner is reminded that the art must be considered as a whole and not piecemeal), is, at best, no more than an invitation to experiment or suggestion of what is obvious to try. This is clear because the combined references do not even provide general guidance as to the particular form of the claimed invention or how to achieve it.

For these reasons it is believed that each and every rejection under 35 U.S.C. § 103(a) must properly be withdrawn and all presently pending claims be allowed. In particular this applies to all claims being rejected based upon the arguments set forth in paragraphs 3, 4, 5 and 6 of the Office Action dated February 25, 2003. In particular this applies to the claims in Subgroups A & B, Groups I, II, III and IV.

c) The Combination of References With De Wachter is not proper

De Wachter (GenBank Accession No. X58052) is nothing more than 18S

rRNA nucleotide sequence information. There are no associated teachings with that reference. Based upon this sequence disclosure the Examiner has opined that: "The nucleic acid of De Wachter is considered to have the property of being suitable for the detection, identification or quantitation of *Dekkera/Bretanomyces bruxellensis*." As noted above, it is the teachings of the references, and not the opinions of the Examiner, upon which a 35 U.S.C. §103(a) rejection must properly rest. More importantly, where the teachings of the art conflict with an Examiner's opinion, clearly the Examiner's opinion cannot be the basis for any rejection.

Kosse teaches that probes **must** be selected to yeast 18S rRNA regions that are fully accessible (**Fact 2**). Kosse also teaches that only the 3' end of 18S rRNA is accessible for species-specific probes (**Facts 21, 22 and 23**). In particular, Kosse teaches that only positions 1682-1728 are "accessible for species-specific probes" (**Fact 21**). By definition, probes for the detection, identification or quantitation of *Dekkera/Bretanomyces bruxellensis* would be species-specific probes. Accordingly, only very little of the sequence information of De Wachter could be considered to have the property of being suitable for the detection, identification or quantitation of *Dekkera/Bretanomyces bruxellensis*. Because the premise of the Examiner's argument is clear error, it is respectfully submitted that the asserted *prima facia* case for the combination of De Wachter with any of the foregoing references is improper and therefore must be withdrawn.

For these reasons it is believed that each and every rejection under 35 U.S.C. § 103(a) based upon a combination with De Wachter must properly be withdrawn. In particular this argument applies to all claims being rejected based upon the arguments set forth in paragraphs 5 and 6 of the Office Action dated February 25, 2003. In particular this applies to the claims in Subgroup B, Groups III and IV.

d) Certain Of The Examiner's Arguments Are Not Well Taken:

In the Advisory Action dated September 29, 2003, the Examiner states that Amann is not being used to reject the present claims. While this is true, the Examiner is reminded that the art must be considered as a whole and not piecemeal. Appellants have provided Amann and take the position that it is the most relevant art with respect to the application of enzyme linked probes for the *in-situ* analysis of yeast 18S rRNA because they appear to be the only persons to actually report the results of such an assay. The Examiner is reminded that Amann and his colleagues **failed to succeed**. Accordingly, with respect to any finding under 35 U.S.C. § 103(a), it is submitted that the Examiner should not attempt to cursorily dismiss the relevance of the Amann reference.

In her rebuttal arguments pertaining to the combination of Kosse and Stender (1998), the Examiner has argued with respect to Amann that: 1) there are no teachings in Amann which indicate that the enzyme linked probes cannot be applied to yeast; 2) Amann provides the results obtained when applying the enzyme-linked probes to the detection of S. cerevisiae; and 3) Amann does not teach that these results apply to the detection of all yeasts (**Fact 4**). Appellants would argue that all of the foregoing statements are misleading and in fact it is believed that the first statement is incorrect. More to the point however, is that the reference needs to be considered as a whole for what it teaches.

With regard to the first statement by the Examiner, it is respectfully submitted that Amann does state: "For now, enzyme-labeled oligonucleotides can therefore **be used only for** specific detection and identification of gram-negative bacteria and several members of *Euryarchaeota* (21)." (Emphasis added, **Fact 8**) This appears to Appellants as a pretty sound retort to the stated position of the Examiner.

With regard to the Examiner's second point, whilst it is correct that Amann provides the results obtained when applying the enzyme-linked probes to the detection of S. cerevisiae, the important point is that Amann **had no success** in

using enzyme-linked probes for such an analysis (**Facts 5, 7 & 8**). Consequently, when considered in view of the experiments with gram-positive bacteria and in the context of the discussions (and knowledge in the art) about how difficult it is to get a molecule with a large molecular weight through the cell wall of gram-positive bacteria and yeast (**Facts 1 & 6**), it is clear that when Amann is considered as a whole, the Examiner's omission causes her statement to be misleading.

With regard to her final statement, it is correct that Amann does not teach that these results apply to the detection of all yeasts. More importantly however, it is also correct that Amann teaches that they have never before (**Fact 5**), and still have not, been able to (**Fact 8**) use enzyme linked probes for the analysis of gram-positive bacteria or yeast. Moreover, Amann cites no literature where such assays have been successful. Accordingly, the ordinary practitioner is without any teaching whatsoever as to how to successfully perform such an assay. More to the point however, is that when such as assay was attempted by Amann and his colleagues, **it failed**.

The Examiner appears to rely substantially on the conclusory statement of Amann for her position (**Fact 9**) with respect to the combination of Kosse and Stender. It is respectfully submitted the conclusory paragraph of Amann amounts to wishful thinking and bald speculation. Most importantly, this statement of a panacea does not undermine the fact that Amann and his colleagues tried to used enzyme-linked probes for the analysis of gram-positive bacteria and yeast **and failed**. More importantly, since 1992, it appears that Amann and his colleagues have not been successful in the asserted endeavor (**Fact 14**) notwithstanding their **optimism** of 1992. Accordingly, little weight should be accorded given to the conclusory statements of Amann.

In the rebuttal arguments presented in the Office Action dated February 25, 2003, the Examiner states: "Thereby, Applicant's have not established that the probe of De Wachter labeled with an enzyme would not be suitable for in-situ

hybridization or other types of hybridization." With all due respect to the Examiner, it is a requirement under 35 U.S.C. § 103(a) that The Office establish a *prima facia* case based upon the teachings of the relevant art. Where a rejection is based upon an Examiner's assertions and the art relied upon by the Examiner does not support the premises of her assertions, it is improper to attempt to shift the burden of proof to Appellant to establish any facts whatsoever. Where the facts do not support a *prima facia* case for obviousness, any rejection is improper and properly must be withdrawn. Accordingly, the Examiner's attempt to shift any evidentiary proof burden to Applicant is simply an irrelevant distraction.

Summary

Because all arguments supporting the final rejection are clear error, it is respectfully requested that this honorable Board of Patent Appeals and Interferences reverse the Examiner's decision in this case and indicate the allowability of all pending claims.

In the event that this paper is not being timely filed, Appellant respectfully petitions for an appropriate extension of time. Any fees for such an extension together with any additional fees that may be due with respect to this paper may be charged to Counsel's Deposit Account 02-3240.

Respectfully submitted
On behalf of Applicants

February 18, 2004
Date:

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9. APPENDIX A

Claims on appeal

1. An enzyme-linked in-*situ* hybridization probe further characterized in that it comprises a probing nucleobase sequence that specifically hybridizes to a yeast specific target sequence.

2. The probe of claim 1, wherein the target sequence is ribosomal RNA.

3. The probe of claim 1, wherein the probe is a nucleic acid.

4. The probe of claim 1, wherein the probe is a peptide nucleic acid.

5. The probe of claim 1, wherein the probing nucleobase sequence is selected to detect, identify or enumerate organisms of one or more species of yeast.

6. The probe of claim 1, wherein the probing nucleobase sequence is selected to detect, identify or enumerate organisms of one or more genus of a yeast.

7. The probe of claim 1, wherein the probing nucleobase sequence is selected to detect, identify or enumerate all yeast in a sample.

8. The probe of claim 1, wherein the enzyme is selected from the group consisting of: a polymerase, alkaline phosphatase, horseradish peroxidase and soy bean peroxidase.

10. An enzyme-linked probe for detecting, identifying or quantitating the

presence of *Dekkera/Brettanomyces* yeast in a sample of interest, wherein the probe comprises a probing nucleobase sequence wherein at least a portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-GGT-GAG-GGT-CGC (Seq. ID No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA (Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.

11. The probe of claim 10, wherein the probing nucleobase sequence is selected to be one hundred percent homologous to a nucleobase sequence identified in the claim.

12. The probe of claim 10, wherein the probe is a peptide nucleic acid.

16. The probe of claim 10, wherein the probe is labeled with soy-bean peroxidase.

18. The probe of claim 10, wherein the probe is support bound.

19. The probe of claim 18, wherein the probe exists attached to an array of probes.

21. A set of enzyme-linked probes for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in a sample of interest, wherein one or more

of the probes comprise a probing nucleobase sequence wherein at least a portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-GGT-GAG-GGT-CGC (Seq. ID No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA (Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.

22. The probe set of claim 21, wherein the probing nucleobase sequences of said one or more probes are selected to be one hundred percent homologous to a nucleobase sequence identified in the claim.

23. The probe set of claim 21, wherein the probe set is specific for both the detection of *Dekkera/Brettanomyces* yeast as well as other organisms of interest in the same sample.

24. The probe set of claim 23, wherein the probes of the set are independently detectable.

25. The probe set of claim 21, wherein some of the probes of the set are blocking probes.

26. The probe set of claim 21, wherein all probes of the set are peptide nucleic acids.

29. The probe set of claim 21, wherein the probes are labeled with the enzyme soy-bean peroxidase.

32. The probe set of claim 21, wherein the probes are support bound.

34. A set of enzyme-linked probes for detecting, identifying or quantitating *Dekkera bruxellensis* yeast in a sample of interest, wherein the two or more probes specific for *Dekkera bruxellensis* yeast comprise a probing nucleobase sequence wherein at least portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5) and CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6) and sequences fully complementary thereto and of the same length.

46. A method for detecting, identifying or enumerating yeast in a sample of interest, said method comprising:

a) contacting one or more species of yeast in the sample with one or more yeast specific enzyme-linked probes, under suitable *in-situ* hybridization conditions, to thereby form one or more probe/target sequence hybrids within the yeast; and

b) detecting enzyme activity within the yeast to thereby determine the presence, absence or number of yeast sought to be detected in the sample.

47. The method of claim 46 further comprising the step of:

c) isolating the yeast using a filter as an isolation medium.

48. The method of claim 47, further comprising the step of:

d) growing the isolated yeast by culture in media.

49. The method of claim 48, wherein the culture is grown directly on the filter, under suitable culture conditions, by placing the filter in contact with media.

61. A method for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in a sample; said method comprising:

a) contacting one or more species of yeast in the sample with one or more *Dekkera/Brettanomyces* yeast specific probes, under suitable hybridization conditions, to thereby form a probe/target sequence hybrid; and

b) detecting the presence, absence or amount of probe/target sequence hybrid and correlating the result with the presence, absence or number of *Dekkera/Brettanomyces* yeast in the sample;

wherein one or more of the *Dekkera/Brettanomyces* yeast specific probes comprise a probing nucleobase sequence wherein at least a portion of the probing nucleobase sequence is at least ninety percent homologous to the nucleobase sequences selected from the group consisting of: AGC-GGG-TCT-ATT-AGA (Seq. ID No. 1); CCA-GGT-GAG-GGT-CGC (Seq. ID No. 2); CGG-TTG-CCC-GAT-TTC (Seq. ID No. 3); TCG-CCT-TCC-TCC-TCT (Seq. ID No. 4); CGG-TCT-CCA-GCG-ATT (Seq. ID No. 5); CAC-AAG-ATG-TCC-GCG (Seq. ID No. 6); GCG-GGC-ACT-AAT-TGA (Seq. ID No. 7); CAT-CCA-CGA-GGA-ACG (Seq. ID No. 8); GTG-TAA-ACC-AGG-TGC (Seq. ID No. 9); ATG-GCT-CCC-AGA-ACC (Seq. ID No. 10) and GAC-AGA-ATC-GAA-GGG (Seq. ID No. 11) and sequences fully complementary thereto and of the same length.

62. The method of claim 61, wherein the probing nucleobase sequences of

said one or more probes are selected to be one hundred percent homologous to a nucleobase sequence identified in the claim.

80. A kit for performing an *in-situ* assay that detects, identifies or enumerates *Dekkera/Brettanomyces* yeast in a sample, wherein said kit comprises:
    a) a filter for isolating yeast from a sample of interest;
    b) culture media for growing the isolated yeast;
    c) fixation solution for fixing grown yeast;
    d) hybridization solution for imposing suitable *in-situ* hybridization conditions;
    e) an enzyme labeled probe specific for detecting, identifying or quantitating *Dekkera/Brettanomyces* yeast in the sample; and
    f) one or more wash solutions for removing undesirable components after performing one or more steps of the assay.

81. The kit of claim 80, wherein the fixation solution and the hybridization solution are the same solution.

82. The kit of claim 80, wherein the soy bean peroxidase labeled probe is a peptide nucleic acid.

83. A method for quantitating slow growing yeast in a liquid sample in less than 48 hours; said method comprising:
    a) filtering a fixed volume of liquid using a filter having a pore size that does not allow the yeast to pass;
    b) incubating the filter containing the yeast, in media and under culture conditions, for 45 or fewer hours to thereby grow microcolonies of yeast;
    c) fixing the microcolonies of yeast to the filter;

d) contacting the microcolonies of yeast with a yeast specific enzyme-linked probe, under suitable *in-situ* hybridization conditions, to thereby form one or more probe/target sequence hybrids within the yeast;

e) detecting enzyme activity within the yeast to thereby determine the presence, absence or number of yeast sought to be detected in the sample; and

f) determining the quantity of yeast in the sample.

84. The method of claim 83, wherein fixing the microcolonies of yeast to the filter and contacting the microcolonies of yeast with a yeast specific enzyme-linked probe are performed simultaneously using a single solution.

85. The method of claim 83, wherein the number of CFU in the sample is determined.

86. The probe set of claim 10, wherein the enzyme is selected from the group consisting of: a polymerase, alkaline phosphatase, horseradish peroxidase and soy bean peroxidase.

87. The probe set of claim 21, wherein the enzyme is selected from the group consisting of: a polymerase, alkaline phosphatase, horseradish peroxidase and soy bean peroxidase.

Appendix B

BP9901-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Serial No:	**09/593,914** **Confirmation No: 8319**
Date Filed:	June 14, 2000
Application Title:	Probes, Probe Sets, Methods And Kits Pertaining To The Detection, Identification And/Or Enumeration Of Yeast; Particularly In Wine
Applicants:	Hyldig-Nielsen et al.
Group Art Unit:	1634
Examiner:	C. Myers
Action Date:	February 25, 2003
Action Type:	Third Office Action On Merits - FINAL
Certified Mail No.:	7003 0500 0000 1731 7079

Certificate of Mailing Pursuant to:
37 C.F.R. § 1.8

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Mail Stop: AF, Commissioner For Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on this 22nd day of August, 2003.

Brian D. Gildea
Reg. No. 39,995

DECLARATION OF DR. HENRIK STENDER
UNDER 37 C.F.R. § 1.132

Commissioner for Patents
Dear Sir or Madam:

I, Dr. Henrik Stender of Fasanhaven 5, DK-2820 Gentofte, Denmark do hereby declare and state that:

1. I am presently employed as Vice President of Research & Development of AdvanDx, Inc. and have been in this position since August 2002;

Appendix B

2. I was formerly employed at Boston Probes & Applied Biosystems from May, 1998 until August, 2002 in various positions, including Director of Microbiology;

3. Before that I was employed at Dako A/S Denmark as Research Scientist from July, 1992 until May, 1998;

4. I received my doctorate from Technical University of Denmark in 1992 in the area of Immunology;

5. I have been employed as a scientist/manager in the field of assay development for a total of 11 years;

6. I am a co-inventor of the above captioned patent application and I have reviewed the claims as currently pending in the application;

7. I have reviewed the Office Action dated February 25, 2003 and the examiners arguments set forth therein;

8. I am a co-inventor of PCT/DK97/00425 (WO98/15648) entitled: "Novel Probes For The Detection Of Mycobacteria", referred to by the Examiner in the above captioned application as Stender (1998) and am familiar with its contents;

9. I have reviewed Kosse et al., Systems. Appl. Microbiol. 20: 468-480 (1997);

10. I have reviewed Amann et al., Applied and Environmental Microbiology 58(9): 3007-3011 (1992);

11. Based upon my review of the Amann et al. reference, I believe that: 1) Amann et al. teach that it was well accepted, at the time of their publication, that enzyme-linked (labeled) probes COULD NOT readily penetrate the cell wall of yeast; 2) Amann et al. had no success with getting enzyme-labeled probes into yeast; and 3) Amann et

al. would tend to dissuade one of skill in the art from attempting to use an enzyme-linked probe to analysis a yeast in an in-situ based assay;

12. Kosse et al. would not, in view of Amann et al., tend to motivate the application of enzyme-linked probes to in-situ assays for yeasts because the reference does not address this particular assay format;

13. Stender (1998) would not, in view of Amann et al. tend to motive the application of enzyme-linked probes to in-situ assays for yeasts because the reference does not address yeasts;

14. One of skill in the art at the time of the present invention would not have a reasonable expectation of successfully applying enzyme-linked probes to the determination of yeast in an in-situ based assay because there was inadequate teaching available as to how to permeablize the cell wall of the yeast to these large molecules.

I further declare that all statements made in this Declaration are true and that all statements made on information and belief are believed to be true. Moreover, these statements were made with the knowledge that willful false statements and the like made by me are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

H Stender

Henrik Stender

8/21 - 2003

Date